

A re-evaluation of the generic assignment of *Bradypodion spinosum* (Matschie, 1892) and some considerations on the genus *Rhampholeon* Günther, 1874

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A re-evaluation of the generic assignment of *Bradypodion spinosum* (Matschie, 1892) and some considerations on the genus *Rhampholeon* Günther, 1874. - The generic assignment of the dwarfed African chameleon *Bradypodion spinosum* is re-evaluated with regard to phenotype, soft tissue morphology, cranial osteology and DNA analysis. All methods used indicate that the species is unequivocally a member of the genus *Rhampholeon* to which it is formally transferred here: *Rhampholeon spinosus* (Matschie) comb. nov. Comparison of DNA with other species of the genus indicates that the genus *Rhampholeon*, as currently composed, is not monophyletic and consists of at least two separate lineages.

Key-words: *Bradypodion spinosum* - *Rhampholeon* - Chamaeleonidae - taxonomy - molecular analysis - Usambara Mountains - Tanzania.

INTRODUCTION

Prior to 1892, only two species of *Rhampholeon* had been described viz. *kerstenii* (Peters, 1868) and *spectrum* (Bucholz, 1874). Although both were initially placed within the genus *Chamaeleo*, Günther erected the genus *Rhampholeon* in 1874 with *spectrum* as the type species for the genus. Both of these species have bicuspid claws. During a stay in the Eastern Usambara station of Derema in 1891, L. Conradt assembled a small collection of mammals and reptiles from which five new species of chameleons were described (Matschie, 1892) including two further new species of *Rhampholeon* named as *Chamaeleon (Brookesia) brevicaudatus* and *Chamaeleon (Brookesia) temporalis*. The latter, although possessing simple claws, has a typical Brookesiinae body habitus and it is not difficult to understand why it was allocated to *Brookesia*. Lastly there was a single specimen of a rather strange small chameleon with simple claws, an unusual ovo-globular rostral projection and prominent spines arranged in irregular rows along the dorsal keel and on the limbs. This chameleon was aptly named *Chamaeleon spinosus*, and all subsequent authors and taxonomists have treated it either as a member of the genus *Chamaeleo* (Werner, 1902; Mertens, 1966),

or more recently as a member of the genus *Bradypodion* [as *B. spinosum*] (Broadley & Howell, 1991; Klaver & Böhme, 1986; Uetz, 2003). However, a number of observations made us question the generic allocation of this species. The available evidence, as well as new characters pertinent to this question, are reviewed below.

MATERIAL AND METHODS

Specimens of *B. spinosum* examined

[BMNH: The Natural History Museum (London, UK), MHNG: Muséum d'Histoire Naturelle de Genève (Geneva, Switzerland), MNHN: Muséum National d'Histoire Naturelle (Paris, France), PEM-R: Port Elizabeth Natural History Museum, (South Africa)].

BMNH 95.4.8.3	(FF), Usambara, Tanzania
BMNH 1974.522	(FF), Mazumbai Estate, Western Usambara, Tanzania
BMNH 1900.12.12.2	(MM), Ambangulu, Eastern Usambara, Tanzania
BMNH 1971.159	(FF), Amani, Eastern Usambara, Tanzania
MHNG 2609.067	(FF), Amani, Eastern Usambara, Tanzania
MHNG 2620.032	(FF), Eastern Usambara, Tanzania (skeleton prepared)
MHNG 2620.033	(FF), Eastern Usambara, Tanzania
MHNG 2620.034	(FF), Mazumbai Estate, Western Usambara, Tanzania
MHNG 2620.035	(MM), Mazumbai Estate, Western Usambara, Tanzania
MHNG 2620.036	(MM), Mazumbai Estate, Western Usambara, Tanzania
PEM-R 5738	(FF), Herkulu estate, Western Usambara Mtns, Tanzania

Material used for the molecular analysis and EMBL/Genbank accession numbers:

Bradypodion tavetanum (Steindachner, 1891)

MHNG 2612.095, Bundunki, Uluguru, Tanzania. (AJ609593)

Bradypodion oxyrhinum Klaver & Böhme, 1988

MHNG 2612.096, Bundunki, Uluguru, Tanzania. (AJ609594)

Bradypodion fischeri (Reichenow, 1887)

MHNG 2609.080, Amani, Eastern Usambaras, Tanzania. (AJ609592)

Bradypodion spinosum (Matschie, 1892)

MHNG 2609.067, Amani, Eastern Usambaras, Tanzania. (AJ609595)

Bradypodion spinosum

MHNG 2620.032, Eastern Usambara, Tanzania. (AJ609600)

Rhampholeon sp.

MHNG 2624.074, Kindoroko FR, North Pares, Tanzania. (AJ609601)

Rhampholeon spectrum (Buchholz, 1874)

MNHN 351I, Cameroon. (AJ609599)

Rhampholeon uluguruensis (Tilbury & Emmrich, 1996)

MHNG 2617.099, Mkungwe, Uluguru, Tanzania. (AJ609602)

Rhampholeon kerstenii (Peters, 1868)

MHNG 2611.010, Captive breeding. (AJ609598)

Rhampholeon brevicaudatus (Matschie, 1892)

MHNG 2609.064, Amani, Eastern Usambaras, Tanzania. (AJ609597)

Sceloporus undulatus (Bosc & Daudin, 1801) (L28075)

For osteological observation, the skeleton of a female specimen from the Museum of Natural History of Geneva (MHNG 2620.032) was cleaned by *Dermestes* sp. larvae for one week, then whitened with H₂O₂.

DNA extractions were made out of liver or muscle samples with the DNeasy Tissue kit (Qiagen) according to the manufacturer instructions. A 0.56kbp fragment of the 16S rDNA was amplified using the universal primers L2510 and H3059 designed by Palumbi *et al.* (1991). PCR conditions were as follows: Initial denaturation 94° for 3 minutes, then (93°, 45 seconds; 55°, 45 seconds; 72°, 1 minute) x 37 cycles, and final extension 72° for 5 minutes. PCR products were checked for size on 1% agarose gel and purified on QIAquick columns (Qiagen). PCR fragments were directly sequenced for both strands using the BigDye cycle sequencing kit (Applied Biosystems), and an ABI 377 automated sequencer. Sequences were aligned with Sequencher™ v4.1.2 (Gene Codes Corp.), and minor corrections were done by hand. The final matrix was analyzed with PAUP* v4.0b10 (Swofford, 2002). Base composition heterogeneity was tested with the same software. Exhaustive parsimony analyses were performed on the whole matrix, and on a reduced dataset comprising the Chamaeleonidae only (without *Sceloporus*) with the following settings: Uninformative characters excluded, characters unordered and unweighted, gaps treated as missing, multistate treated as uncertainty. Branch support was estimated with 10'000 bootstrap pseudoreplicates.

RESULTS AND DISCUSSION

MORPHO-ANATOMICAL AND ECOLOGICAL OBSERVATIONS

Bradypodion spinosum is a dwarfed, short tailed, cryptically coloured, and rare inhabitant of the Afro-montane forests of the Eastern and Western Usambara Mountains of northern Tanzania (Fig. 1). The chameleons live in the lower strata of the deep forest biotope where they may be found at perch heights from a few cm to 3 meters elevation. Over part of their distribution (in the Eastern Usambaras) *B. spinosum* is sympatric with *Rhampholeon temporalis*. A superficial glance at this chameleon reveals some features that are not seen in the typical *Rhampholeon*. It has a network of interstitial skin that divides the flanks into clumps or islands of tubercles. In the genus *Rhampholeon*, this character is only seen in *R. gorongosae* Broadley, 1971. The tubercles on the flanks are not composed of sheets of stellate tubercles as is seen in other species of *Rhampholeon*, but rather of polygonal tubercles. The occiput of the skull has a rather prominent casque and the tail is relatively long for the typical *Rhampholeon*, averaging around 40% of the total length. It also has simple claws, a feature common to all species of the *Chamaeleo* and *Bradypodion* genera and to date only described in *Rhampholeon temporalis*.



FIG. 1

Rhampholeon spinosus comb. n., female. Herkulu Tea Estate, Western Usambaras, Tanzania. (Photo. C. Tilbury)

Bradypodion spinosum has however some morphological features unlike any seen in the genus *Bradypodion* (sensu lato) but reminding one of *Rhampholeon* (sensu lato). A clearly demarcated ridge of inter-orbital tubercles is present. In addition, the gular region is adorned with posteriorly diverging rows of low conical tubercles. Whilst there are a few species of *Chamaeleo* (e.g. *tempeli*, *laterispinis*) and one species of *Rhampholeon* (*kerstenii*) that demonstrate this gular arrangement, there are no members of the east African *Bradypodion* that have any trace of gular ornamentation. Closer examination of the scalation of the lower flanks shows that in areas there are small zones of stellate tubercles. In addition the rostral process is unlike any other rostral processes seen outside the *Rhampholeon* Type II group (Rieppel, 1987). Where they occur among the genus, the rostral process is formed by a cluster of small conical tubercles around a soft tissue cushion that projects forward off the snout as a flexible process. Occasional specimens of *spinosum* may also have weak axillary dermal dimples equivalent to the better formed mite pockets of other species of *Rhampholeon*, and finally all specimens examined have low palmar and plantar spines. These are only seen in the *Rhampholeon* type II species (sensu Rieppel, 1987).

Klaver (1979) provided a valuable insight into the relationship of *spinosum* to the Brookesiinae when he commented that the simple sac-like a-diverticulate lungs of *spinosum* were "truly *Rhampholeon* like". A single male specimen of *spinosum* (MHNG 2620.036) was found with everted hemipenes. These demonstrate an acalyculate hemipenal truncus and a complete absence of any apical structures – a condition not described outside the Brookesiinae genera. It is possible that the hemipenes are not fully everted and so further comment on the apex is withheld.

OSTEOLOGICAL CHARACTERS

The skull of *Bradypodion spinosum* demonstrated the following osteological features. The nasals are paired and form the dorsal margin of the external nares. The nasal process of the pre-maxilla meets the frontal bone just anteriorly to the nasals. The external nares reach the frontal bone. There is no fontanelle between the nasal and the prefrontal. The parietal is triangular and resembles that of *Rhampholeon platyceps* as illustrated by Rieppel (1987, Fig. 9E). The latter bone clearly lacks a supratemporal

process, but the squamosal presents a normal dorsal process closing the upper temporal fossa posteriorly. These characters were all used by Rieppel (1987) to define taxa of the *Rhampholeon* type II group. The supratemporal itself is absent from the specimen we observed. Although this bone is present in most of the *Rhampholeon* type II species, exceptions occur like in *R. spectrum* (Rieppel, 1987). The presence of the splenial could not be ascertained. Additionally it was observed that the orbitae are bordered by three bones (i.e. including the frontal), a character typical of the Brookesiinae according to Klaver & Böhme (1986).

MOLECULAR PHYLOGENY

Partial DNA sequences from the 16S mitochondrial rDNA were obtained for 10 taxa and aligned with an outgroup belonging to the Iguanidae (Phrynosomatinae). Out of 558 aligned characters, 62 were excluded for alignment difficulties and, among the 496 remaining ones, 82 were parsimony informative (70 when *Sceloporus* was excluded). No compositional bias was detected in the dataset. Pairwise uncorrected distances between the outgroup and ingroup taxa vary between 22.3 and 25.8%. Interspecific distances within the ingroup fluctuate between 4.3 and 13.7%. There are 5 substitutions (4 transitions and 1 transversion), representing 1% divergence between both *B. spinosum* samples. The parsimony analysis of the 10 chameleons sequences resulted in a single most parsimonious tree (Fig. 2) with a length of 158 (CI 0.601, RI 0.606). In this tree *B. spinosum* is the sister-group of *Rampholeon* sp. a new species from the neighbouring North Pare Mountains. This relationship is well supported. Both species forms a monophyletic group with two *Rhampholeon* (II) taxa although bootstrap support is much lower for this node. Interestingly this group of species includes *R. spectrum*, the type species of *Rhampholeon*. The other *Rhampholeon* (belonging to type I) group together, and so do the *Bradypodion* taxa, however all these nodes are weakly supported by bootstrap values. When the tree is rooted with the *Sceloporus* sequence, its topology remains the same and the root branches between the *Rhampholeon* (type II) and the *Bradypodion/Rhampholeon* (type I) clades (Fig. 2). However, because of saturation concerns with the use of the available outgroup we prefer to discuss our results on the basis of an unrooted tree. In any case this does not change the relationships of *B. spinosum* with *Rhampholeon*. Despite the obvious caution we should exercise because of the weak bootstrap support for some branches, these additional molecular results confirm the conclusions we derived from morpho-anatomical analyses. They allow us to obtain two important conclusions: a) *B. spinosum* is definitely more closely related to (some) *Rhampholeon* species than to other *Bradypodion*, therefore its transfer to *Rhampholeon* is fully justified; and b) The genus *Rhampholeon* is most probably not monophyletic and comprises at least 2 separate lineages concordant with the Type I and Type II groupings. This idea has already been suggested by several authors on the basis of diverse morphological characters (Bauer, 1997; Rieppel, 1987; Tilbury, 1992; Tilbury & Emmrich, 1996, Townsend & Larson, 2002), and is now supported by a new class of characters. The taxonomic decisions and corresponding nomenclatural acts needed to reflect this situation will be published elsewhere on the basis of a more comprehensive dataset (Matthee *et al.*, in prep), except for the new combination proposed below.

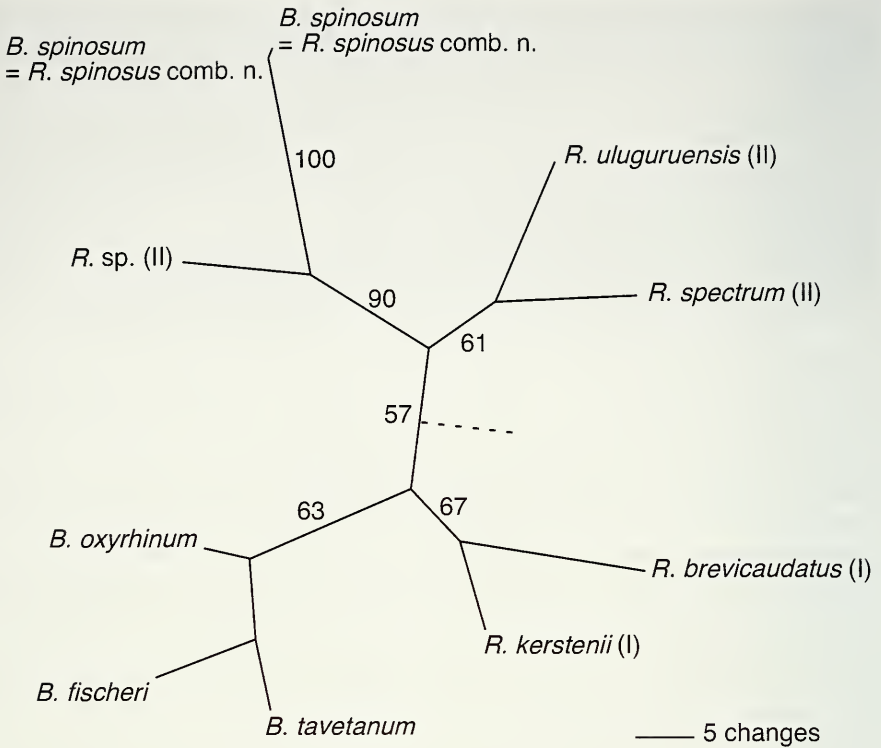


FIG. 2

Unrooted phylogram showing the phylogenetic relationships of *Rhampholeon spinosus* comb. n. based on a parsimony analysis of partial 16S rDNA sequences. Numbers on branches are bootstrap values (10'000 repeats). *B* = *Bradypodion*, *R* = *Rhampholeon*, (I) = *Rhampholeon* type I, (II) = *Rhampholeon* type II. The dotted line indicates the position of the root when *Sceloporus undulatus* is used as outgroup.

CONCLUSIONS

On the basis of its general phenotype, rostral process, simple lung morphology, presence of accessory palmar spines, row of enlarged inter-orbital tubercles, acalyculate hemipenial truncus, osteological skull structure, and particular DNA characteristics, *B. spinosum* clearly does not belong in the genus *Bradypodion* (sensu lato), but rather in the genus *Rhampholeon* [Type II (Rieppel, 1984; Tilbury, 1992)]. Whilst it is apparent that *spinosum* belongs within the Brookesiinae subfamily, its relationships within the genus *Rhampholeon* are by no means clear yet. By virtue of its acalyculate hemipenial truncus it is likely to be more closely related to the species around *R. boulengeri* and *uluguruensis*. Compared to the sympatric species *R. temporalis*, the exaggerated development of the rostral process, the small gular spines and the flank scalation of *spinosum*, more reminiscent of the other chameleon genera, may indicate differentiation due to character displacement (Brown & Wilson, 1956). The gular spines are very reminiscent of those found in *R. kerstenii*. The flank scalation, and

simple claws of *spinosum* are morphological features that appear to bridge the generic gap between *Rhampholeon* and *Chamaeleo* and may be important clues in the complex evolutionary history of the Chamaeleonidae. The molecular data reported here do not allow us to confirm this assumption, however preliminary analyses of more complete molecular datasets tend to support this idea (JM, unpublished data).

We conclude with the transfer of *B. spinosum* to the genus *Rhampholeon* Günther, 1874, and propose the following new combination: *Rhampholeon spinosus* (Matschie, 1852) **comb. nov.**

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